

1/11/55

Irradiation eff'd = WCG-1-S. Used overnight aerated culture of grown in Ag I broth, rm temp.

Irradiated 10^{-4} dilution; cfzd, washed, resuspended in saline, then diluted.

90 see irradiation = big camp.

Diluted further to give 10^{-7} and 5×10^{-7} dilutions of original culture on plates.

Inc. 25° incubator

1/13/55

9:00 AM: Irradiated culture plated 1/11: Dilutions used too high: $5 \times 10^{-7} \rightarrow$ about 20 / plate. Colonies still very small; inc. another day before replicating.

Started culture for second irradiation: 0.1 ml Ag I broth culture (not aerated) \rightarrow Ag I broth aerated, rm temp.

1/14/55

Replicated 5 plates of 5×10^{-7} dilution to minimal. 6th plate contaminated, sick molds; pick colonies to complete for later replication. Also pick the few colonies from 10^{-7} dilution to complete.

1/15/55

Empired plates repl. to min from 1/11 irradiation: 9 possible autotrophs from 245 colonies. Pick, spot on complete. Some colonies very rough; use caution in identity. If possible, concentrate on typical colonies.

Second UV run, WCG-1-S:

Culture: Aerated at run temp overnight; stored
in aeration at run temp another 24 hrs.

Cfcd; pellet washed in saline; diluted to 10 ml in
saline. 10^{-4} dilution irradiated 90 sec.

Plating: 0.1 ml and 0.2 ml 5×10^{-6} dilution.

1/17/55

Second UV run: Very few colonies visible at 48 hrs!

Continue incubation at 25°. By 2:30 pm colonies on
1% dilution large enough to count. Total no. of colonies = 2. Avg
 5×10^4 col. per plate.

"Autos" from first run: Of the 9 spotted on complete,
three are gummy; look like typical Agrobacterium
growth. Other six rough & rather battery. Replicate
all to mineral. (Plates which were contaminated or
too dilute for replication)

Of colo. picked from 1st run plates & respotted, then repl to
min., 1 of 66 appears to be an autotroph. Test
along w/ other 9.

Made up \approx 2 mineral tubes. To slant medium, mix 1 drop
water suspension from WCG-1-S stab into 1) complete, aerated
Used 1 drop 1% sterile Na_2S /tube (10 ml) 2) min + Na_2S aerated
3) " " " not aerated

1/18/55

"Autos" from first UV run: 4 of 10 grew well on
min. in 24 hrs. These included all the 3 "typical"
gummy growers. 2 of the rough type show poor

growth on ^{replica} ~~replication~~ both to min & complete at 24 hrs.
 Remaining 2 replicates show good growth on complete,
 poor growth on min. The 10th "cups", brushed
 on plates to which others were replicated, shows fair
 growth on compd at 24 hrs, no growth on min.
 Continue incubation of these plates until early pm.

~~Several~~ Two of the "rough" spots \rightarrow mixed
 growth when replicated to min & complete; center
 of spot rough sparse growth; outer ring of heavier,
 gummy growth. Especially marked on complete.
 So it appears these rough colonies may be Cystobacterium.

Colo picked from 2nd UV: Overnight growth
 poor; continue incubation until early pm before
 replicating to min.

More colonies now visible (3 days) on 5×10^{-4} & on
 10^{-7} plates from 2nd UV run. Rich; spot on
 complete for repl. to min.

Check on Ag 2 minimal: No growth overnight in
 min tubes & or 5 aeration. Incubated Ag 1 tube
 (compd) slightly turbid. Run temp probably quite
 low during night.

1/19/55

Check of minimal liquid (see 1/17): At 2 days, very good growth of WCST-1-S in min (\pm Na₂S) aerated. About the same turbidity in un aerated minimal as in un aerated complete.

Why Na₂S & aeration needed for best growth?

Seems contradictory. Effect of aeration may be largely agitation rather than O₂ availability; but at pH slightly below 7 should thiamine sulphide would be rapidly lost.

Set up: min + Na₂S } aerated
min \mp Na₂S }
min \mp Na₂S } un aerated
min \mp Na₂S }

1/20/55

Possible errors from 1st UV run: The rough type grew up eventually on minimal. Check these on a couple of other sugars; could poor growth & lack of germ be due to mutation involving utilization of sucrose?

* 10 picked from first run appears to be anastomoph. Stab; prepare random tubes. Growth is rather scant even on complete. How be sure of identity ????

Second UV run: Of 53 col. picked, 10 are poorly growing, rough type. If this is a contaminant, it certainly appears

consistently.

One possible aspect from 2nd run. Stab,
streak or complete.

1/21/55

Effect of aeration \bar{c} & \pm Na_2S : (48 hrs)

Not aerated: Little difference \bar{c} & \pm Na_2S . Poor growth.

Aerated, no Na_2S : better growth than \pm aeration.

Aerated, \bar{c} Na_2S : Very good growth; ad. $10^9/\text{ml}$.

1/22/55

Inoc Ag 2 roundouts \bar{c} UV1+10, UV2-1, and WCG15
from water suspensions from stabs.

UV2-1 grows very poorly when streaked on complete.

"Rough" strains streaked on compl. \bar{c} glucose & mannitol
look the same as on sucrose.

1/24/55

Roundouts (2 days)

	<u>WCG15</u>	<u>UV1-10</u>	<u>UV2-1</u>		<u>WCG15</u>	<u>UV1-10</u>	<u>UV2-1</u>
O	++	-	-	HC	++	+	-
A1	++	-	-	YNA	+	\pm	-
A2	++	-	-	VITS	++	-	-
A3	++	-	-	compl	++	++	++
A4	++	-	-				
A5	++	-	-				

1/25/55

Randoms (3 days)

	WCGIS	1-10	2-1		WCGIS	1-10	2-1
0	+++	-	-	AA5	++	-	-
AA1	++	-	-	HC	++	++	++
2	++	-	-	YVA	++	-	+
3	++	-	-	VITS	+++	-	-
4	++	-	-	compl	++++	+++	+++

Dry amino acid single omissions. [The HC used is contaminated, so growth in HC tubes not necessarily due to HC components].

UVnum (#3)

24 hr aerated culture (A_g , room temp)

90 sec. . Pl. 0.1 + 0.2 ml 10^{-5} dil. (Irradiated 10^{-4})

1/28/55

UV 3: Replicated to minivid from plates = >10 col. Picked from plate = <10 col & spotted on compl.

One yellow (or creamy) colony. Picked to stab, labelled UV 3-1. Colony was same size, shape, height, & texture as others on plate. Check motility; growth = \pm suspended, \pm aeration.

1/28/55

Rundnons, single omissions of AA groups (2 days)

	<u>UV1-10</u>	<u>2-1</u>		<u>1-10</u>		<u>2-1</u>	
0	0	0	-5	+		+	
-1	+	+	all	+		+	
-2	+	+	all + vits	+		+	
-3	+	+	HC	-		+	fresh AC used (taken from freezer)
-4	+	+	cetyl	++		++	

Very similar to behavior of previous mutants. New vit mix not inhibitory. Try various carbon sources & N sources

1/29/55

Rundnons, single omissions, 3 days

	<u>UV1-10</u>	<u>2-1</u>		<u>1-10</u>		<u>2-1</u>	
0	0	0	-5	++		+	
-1	++	+	all	+++		+	
-2	++	+	all + vits	+++		++	
-3	++	+	HC	+		++	
-4	++	+	cetyl	+++		++	

3rd UV run: Replicated to mini. col. picked to complete 1/28.

Direct replication: 2 possible auxos out of ca 80 col. One is the yellow colony.

1/31/55

UV3: Col. picked, repl. to mini 1/29: 2 possible auxos (both rough, so possibly just poor replication). { UV3-3 + UV3-4 }

Screen as follows: 1) min. 2) min + HC. 3) min + all AA groups together. 4) As 3, + VITS. 5) YNA.

Also include UV3-1 + 3-2 in tests.

2/2/55

Rendowns of UV3 apos: 2 days

	<u>HC</u>	<u>AA</u>	<u>AAV</u>	<u>YNA</u>	<u>Ag I</u>	<u>O</u>
3-1	++	++	++	+	++	-
2	+	++	++	++	++	-
3	±	+	-	-	++	±
4	-	-	-	-	++	-

2/3/55

UV3 rendowns, 3 days.

	<u>O</u>	<u>HC</u>	<u>AA</u>	<u>AAV</u>	<u>YNA</u>	<u>Ag I</u>	<i>These were left for next day; no further changes in other tubes, no difference in other tubes</i>
3-1	±	+++	+++	+++	++	+++	
2	±	++	++	++	++	+++	
3	±	++	+++	-	-	+++	
4	-	-	-	-	-	+++	

2/8/55

Inoc. AA rendowns of UV3-2 & 3-3. (Inoc., single addition)

2/9/55

Set up rendowns of UV3-4 as follows: HC, HC+V, HC+YNA, YNA, YNA+V, V, HC+YNA+V, YX. Brew only in YX

2/10/55

Rundowns, UV 3-2 + 3-3 (2 days)

	<u>3-2</u>	<u>3-3</u>		<u>3-2</u>	<u>3-3</u>
O	-	-	A4	-	-
A1	-	-	A5	-	-
A2	-	-	XAS	+	not included
A3	-	-	compl + its compl.	not included ++	to check for traces +++

If these results check out after another 24 hrs, try 3-2 = individual vits + 3-3 = AA single omissions

2/12/55

Set up rundown = individual vits = UV 3-3. (B-12 may be contaminated).

2/13/55

Incub UV 3-2 vit rundown.

2/14/55 UV 3-2

Vit rundown; At about 28 hrs, fair growth in vits mix,
trace of growth in biotin.

Would pay to do individual vit = all autos; behavior has been
constant in vit requirement, but mix may be toxic to some.

Also: 3-3 = ~~long~~ AA groups.

2/15/55 Vits, UV 3-2: Fair growth in biotin; other vits O

WCA

UV3-3 inoc. random, A & groups.

2/17/55

UV3-3 no growth in D&G groups or in all AA's (2 days)

2/20/55

UV1-10 & 2-1 in vits: no growth in individual vits or mix. (Inoc. 2/18)

2/22/55

UV1-10 No growth in individual vits, but some growth in vit. mix (4 days) May have double requirement, or conc. of regu. vit in individual tube may be too high, since some are higher than in mix. Also may be reversal - see UV2-1

UV2-1 Growth in the following tubes (very slight)

Riboflavin	paba	
B-12	vit mix	Reversions?
Pantothenate		Dirty tubes?

2/23/55 (5 days)

UV1-10 Growth in: Biotin, choline, ni, paba, vit mix

UV2-1 " " : trace in those listed previously. ~~Very~~ Very scant even in vit mix.

2/24/55

Set up min, min + glycine (1 mg/10 ml), min + vits, & min, vits, & glycine to see whether glycine will speed growth in vits & itself permitting growth (1-10 & 2-1)

WCG Jnl. 2/25

3/1/55

UV 1-10 & 2-1 in glycine, vits.

at 3 days, 1-10 ± in glycine, glycine + vits.

4 days 0 -

glyc ±

vits +

vits+gl ++

2-1 at 4 days: 0 -

glyc +

vits +

vits+glyc - (!?!)

3/4/55

UV 3-3 (2 days) 3 days

0 - - A4 - -

A1 ++ ++ A5 - ++

A2 - - all AA's - ±

A3 - + comple +++ +++

3/6/55

(UV 3-3)

A1 renditions in 3/4. Possibly trace of growth in lysine

3/7/55

A1 renditions - 3 days (at room temp; cold at night)

0 - Ruth -

Lys + A1 -

Arg - comple ++

Cyst ±

Try 2 & 3 lysine & aeration. Usually Lys - showed some more clear-cut results because of more rapid growth.

WCG

3/8/55

Start culture of WCG 1^(S) for another UV exp^l (≈ 4) to try to get a readily identifiable auxotroph.

Ag 1, room temp, aeration.

1) 2 ml v.
2 + 1 ml

TRANSFER STAGES

3/10/55

Irradiated 90 sec. (10^{-4} dil)

Excellent growth of WCG 1-S for irradiation. Plated 0.2 ml/plate of 2×10^{-5} dilution (Ag 1 medium)

UV 3-3 in min, lbg, Ag 1 aerated; In 3 days, faint growth in all tubes.

3/14/55

UV 4 : 6 possible auxotrophs - 1 is ~~the~~ a light yellow similar to UV 3-1.

3/15/55

UV 4-1 (yellow) is an auxotroph; all others grew in min.

3/18/55

UV 5 : 48 (approx) hr culture, Ag 1 broth, aerated. Irradiated 10^{-4} dilution for 90 sec. Plated 0.1 ml/plate of 10^{-5} dilution. Inc. 25°.

3/19/55

UV 4-1 & UV 3-1 (yellow): Striated or complete. 3-1 rough, 4-1 smooth. Appearance \cong rough & smooth WCA-1 except for color. Smell like Cyanotheicum.

Try running down requirements(s)

Spread 4-1 broth (5 days old) on minimal; look for possible "reversions" to prototrophy which should appear as papillae. If any, what color? (I.e. is yellow color result of a metabolic block.)

Also - compare growth in complete broth \pm 5 aeration \pm WCA-1.

3/21/55

UV 3-1 & 4-1 Both show scanty growth in broth ^{complete} 5 aeration, good growth aerated (but not so heavy as WCA-1) 4-1 spread on minimal - No papillae at 2 days.

Rundowns:

UV 3-1 ++ in A4, + in A5, ± in others } 2 days
UV 4-1 0 in all rundown tubes.

3/22/55

UV 3-1 & 4-1 rundown

4-1 no growth except in complete

3-1	A1	0	A5	++	Top: 1 d. old at
	A2	0	VTS	++	Comp. top of A4:A5
	A3	0	0	0	(4) best, tan, no, glut prot esp 5) along edges, ser, O/H prot
	A4	++	compl.	++	

WCA

3/24/55

UV 5: Of 7 possible cups picked, all show some growth in
min at 2 days. Two have only very faint turbidity; keep these,
check especially for vits. ~~but second check smaller specimen, no
growth in 4 days~~ UV 5-1 & 5-2

(3/19)

UV 4-1 Spread on Ag 2 plate: 1 papilla, which appeared
in about 3 days. Appears to be slightly buff-colored. Pick, streak
on complete, check on min for color & pistrophy.

3/26/55

UV 3-1 streaks - 3 days (run temp - cold at night)

B1	0	pyri	0	A4	++	A5	±
ribo	0	pent	0	hist	0	alan	0
nic	0	<u>chol</u>	++	threo	0	<u>glyc</u>	±
paba	0	vits mix	0	glut	0	ser	0
bio	0			prol	0	<u>OHP</u>	+++
PGA	0			<u>aspart</u>	++		
Gall p. magnif. 1/2 nos						compl	+++
K	0						

Streak out; recheck individual colonies.

3/28/55

Papilla from UV 4-1 - picked from min, streaked on
complete. Colonies yellow. Pick several, check for
anastrophy.

MCB

3/31/55

UV3-1 : Randoms inc. from 2 individual col. (2 days)

	(1)	(2)		(1)	(2)	(3 days)
o	0 0	0 ±	A4	0 0	++	+++
vits	0 +	++ +++	hist	0 0	0	+
chol.	0 0	0 +	threo	0 0	0	0
inos	0 0	0 0	gluta.	0 0	0	++
A5	+ +++	0 ±	prol.	0 0	+	+++
alan	± ++	0 +	aspark	0 0	++	+++
glyc	0 0	0 +++	cyste	+++	+++	
Ser	0 0	0 0				
OHP	0 0	0 +				

Indicates erratic results prob due to
revisions?

5-1 & 5-2 randoms: grew in min. However, there are apparently some inhibitions.

	<u>5-1</u>	<u>5-2</u>
o	+++	++
A1	+	±
A2	0	+
A3	±	0
A4	+++	++++
A5	0	0
Vits	+++	++++

4/2/55

Col. from papilla from UV4-1; autotroph: However, 1 streak -> faint growth after 5 days, ~ 1 large colony, many small. On min, large col appears white. Hist, streaks are complete.

4/5/55

(cont. 3/29)

Final comparison of randoons on two col. from UV 3-1

	(1)	(2)		(1)	(2)
O	±	+	A4	+	++
vits	±	+++	hist	++	· +
chol	±	++	threo	0	±
inos	±	+++	glut.a.	++	++
A5	++++	+++	prol	+++	+++
alan	+++	++++	aspart	++	++
glyc	++	++++			
ser	0	+++			
OHP	±	+++			

UV 4-1 Picked from large col. on mini: Streak mixed; yellow col as before, also a white, rough col. type - latter extremely rough - probably a contaminant.

Might try plating UV 4-1 or 3-1 together \pm WCG 16. Main purpose would be to look for colonies \pm yellow & white sectors. First check nutrition of WCG 16; also make sure UV 4-1 (or 3-1) does not respond to biotin.

4/12/55

Spread water suspensions (from slants) of WCG 16 & UV 4-1 on plates of mini \pm biotin: Also cross-brushed ear. \pm SAG.

4/14/55

WCG 16 - confluent growth on mini \pm bio; none \pm bio.
UV 4-1 - no growth on either plate

WCG 16 5^S
UV 4-1 5^R

WCG

4/22/55

Results of attempted cross of WCG 16 & UV-4-1 (4 days 25°)

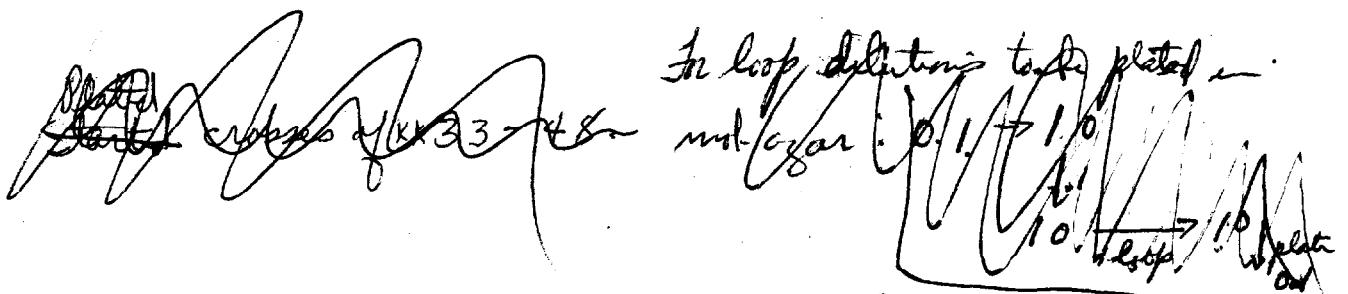
Controls: WCG 16 can grow \pm SM at 200 $\mu\text{g}/\text{ml}$
" " no growth \pm brother.

UV 4-1 Some background \mp SM, none \pm SM,
though culture appeared to be S^R

With mixtures, confluent growth on all media. Growth
is white, i.e. WCG 16

$\begin{matrix} \text{min} \\ \text{min} \\ \text{min} + \text{SM} \\ \text{min} + \text{SM} + \text{brother} \end{matrix}$

3-3 AA groups



1-10 HC~~+~~- ; in AA single group omission, grew well in all tubes except min control. AA₈- strong addition all - ; late growth (4 day) in some vts.

2-1 HC+ ; poor growth in all AA single omission tubes.
late growth (4 days) in some vts.

3-1 (yellow) HC+ ; AA's together + ; poor in YNA

3-2 HC faint +, AA's together +, better in AAV ; good YNA
Biotin

3-3 HC+, AA's together strong +, AAV- ; YNA-

3-4 Shows only in complete or min + YX
(Have tried HC, HCV, HCYNA, YNA, YNAV, V, HCYNAV)

Possible schemes of mixing WCG16 & UV4-1

See whether both lines < less 5%.

If could obtain:

yellow S^R x- B+
and S^S x+ B-

Plate on

1) min

2) min + YX

3) min + biotin + YX

What could grow?

all x+ B+

only x+ B+ S^R

all x+ S^R

/ undiluted

color, S

color

color, B

Lysing with Cys